Karr.

Studies On The Formation And Distribution Urea And Uric Acid In Vertebrates.

STUDIES ON THE FORMATION AND DISTRIBUTION OF UREA AND URIC ACID IN VERTEBRATES

The Urea Content of the Blood and Tissues in Vertebrates under Normal Conditions and after Inanition and Restricted Diets

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY WALTER GERALD KARR

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General Introduction.

The problem of protein metabolism and protein requirements of the organism are today among the most absorbing and fundamental in the whole realm of physiological chemistry. Closely associated with these problems is the problem of the formation and distribution of urea,since this has long been recognized as the chief end-product of protein metabolism in the higher vertebrates,with the exception of birds and reptiles,in which its place as the chief nitrogenous end-product of metabolism is taken by the more complex uric acid. Altho much work has been done on the relation of urea to the intermediary metabolism of protein and amino acids,many problems remain to be solved,the solution of which is now a possibility with the more suitable and accurate methods of analysis developed in the last few years. The site of the formation of urea has not been determined,whether its synthesis be a function of the liver only,or one of all the tissues. The role of urea in the metabolism of birds is not well understood,nor is the significance of the variations of the urea content of the blood and tissues clearly defined. In view of this it would seem of value to make a comparative study of the urea in the blood and tissues of several species of vertebrates,including also those animals whose final end-product of nitrogen metabolism is not urea. The influence of starvation and certain restricted diets on the urea content of blood and tissues has also been investigated.

Methods for the Determination of Urea.

Many of our recent developments in the knowledge of the role of urea in intermediary metabolism are due to the perfection of the methods for the determination of small amounts of urea. Older methods were disadvantageous in that they required rather large amounts of blood,which necessitated experiments on large animals,and the death of the animal to obtain sufficient blood for analysis. They were also inaccurate,as our present methods show us,in that either some of the urea was lost in the removal from the solution of interfering substances,or that other substances were broken down and their decomposition products determined as urea. Of our present methods,those of Folin (1) and Benedict (2) have generally given satisfactory results in urine and other macro-analyses. But since they are known to break down the allantoin and a small part of the uric acid,creatinine,etc.,quantities which are negligible in a macro-analysis,errors in the determination of small amounts are thereby introduced which render the methods of little value in tissue analysis. The most satisfactory method at present seems to be the urease method of Marshall (3) as modified by VanSlyke & Cullen (4). The urease method is entirely specific for urea,also short and easy of operation,and with Folin's (5) aeration method for ammonia makes a very suitable and accurate determination of urea. Moreover,no removal of protein or other substance is necessary,thus avoiding loss in that respect.

The Site of Urea Formation in the Organism.

As was stated above,the site of urea formation in the body is very uncertain. Our present idea is that urea is formed from ammonia and carbon dioxide. The ammonia is regarded as being de-

rived from deaminization of amino acids set free in protein catabolism. Hence in consideration of the problem of urea formation two aspects must be considered,deaminization and formation of urea from ammonia and carbon dioxide. All data seem to show that urea can be formed from ammonia in the liver,but there is still open the question as to whether this synthesis is exclusively a function of the liver or whether it is shared by all the tissues.

In support of the view that the liver is the chief place where amino acids are converted into urea is the classical work of Salaskin (6) in which he perfused livers with amino acids and obtained a large increase of the urea content in the blood,as well as an increase in the urea from the perfusion of ammonium salts. Leschke(7) fixed sections of liver with mercuric nitrate and stained them with hydrogen sulfid. He found that during the height of digestion,and on injection of amino acids or ammonium salts that the cells stained deeply in each case whereas in inanition there was no staining except in the Kupffer cells which held the stain on the basis of which he assumed that these cells have some special function in urea formation or regulation. Jansen (8) perfusing livers with amino acids confirmed the previous experiments and obtained notable increases in urea. (From 26 livers weighing 45-315 gms. he obtained 29-522 mgms. of urea in one hour perfusion). Van Slyke,Cullen & McLean (9) showed that at various intervals after feeding protein the urea content of blood from the hepatic vein was 3-20% higher than that of the portal blood,also that the increase of the urea content of blood passing thru muscle did not occur. These experiments seem to indicate that the liver has some special function in the formation of urea from the amino acids.

In support of the contention that urea formation is a general

function of body tissue is the work of Taylor and Lewis (10) who removed the entire alimentary tract in order to rule out the increased ammonia content of the portal blood which,as shown by Folin and Denis (11),has its origin in bacterial processes of the intestine and not from protein metabolism within the body. The determination of the non-protein-nitrogen and urea content of the hepatic and peripheral blood showed the same quantities in each or a very slight excess in the hepatic blood (Average gms. urea-N. per 100cc.,hepatic- .0128, peripheral- .0108) from which it was inferred that the liver does not predominate greatly over the other tissues in urea formation.

The principal evidence of the inability of the liver to deaminize amino acids is the work of Fiske and Karsner (12) who perfused livers with ammonium salts and amino acids,obtaining an increase in urea with the ammonium salts but not with the amino acids. Jansen(8) has criticized these perfusion experiments with negative results in that the blood was not sufficiently arterialized during the perfusion Fiske & Sumner (13) removed the liver from the circulation by ligating the blood vessels and on injecting amino acids into the blood stream obtained as great an accumulation of urea in the blood and tissues as was obtained when the hepatic circulation was intact. From these experiments they concluded that the liver cannot be the chief cource of urea formation and that all the tissues must share the function. It should be borne in mind,however,that the urea formation must be a function of the cells - all cells probably. It is to be anticipated then that the richly cellular organs,of which the liver is the largest of the body,would yield larger amounts of urea, weight for weight,on perfusion than the less cellular organs,e.g. muscles. This point of view may help to reconcile the apparently contradictory evidence just cited.

Distribution of Urea in the Organism.

On the distribution of urea in the animal organism many figures are available, but due to the inaccuracy of the older methods of determination, the figures of the last few years only would seem to be trustworthy. Schöndorff (14) in 1899 reported a large number of figures on the urea content of tissues, but, due to the methods used, these are all apparently too high. He showed however that the distribution of urea was general in the body and the quantities approximately equal in different tissues, altho his figures are not very consistent on this point. Marshall and Davis (15) have given an accurate method for urea analysis in tissues by means of the enzyme urease. They have shown that in the dog urea is distributely approximately equally in all the tissues of the organism with the exception of the fatty tissues, kidneys and urinary tract, that the figures for normal dogs vary from 18-31 mgm. urea per 100cc or gms., and that on injection of urea into the blood stream it is absorbed very rapidly, only about 10% being left at the end of the injection. (The low content of urea in fatty tissues is due to the low content of water and the consequent decreased power of absorbing the urea, while the high content of the kidneys and urinary tract is due to the saturation with urea which is in the process of excretion from the body.) Folin and Denis (16) give figures on the urea content of the blood of different species showing that it varies with the species. The same observers find the urea content of human blood quite constant at 11-13 mgm. urea N. per 100cc. Schwartz & McGill (17) report figures from fifteen different authors on the urea content of human blood showing a variation from 11-25 mgm. urea N. per 100cc. McLean & Selton (18) find the urea content to vary with a number of factors, especially the nature of the diet, giving figures from 10-23 mgm. urea N. per 100cc.

Addis and Watanabe (19) in analyzing human blood for urea found marked variations in different individuals and showed that there was little relation between the concentration of urea in the blood and the amount excreted in the urine. Denis (20) analyzed the blood of the elasmobranch fish (dog fish,sand shark and skate) and found the urea content to be much higher than that of mammals (800-1000 mgm urea per 100cc blood). The urea content of the blood of the teleosts (goose fish) on the contrary was lower than that of mammals,being about the same as that found by Folin and Denis (16) for chickens (8-9 mgm urea N per 100cc blood). The urea content of the blood of other fish was about the same as that of mammals. Van Schröder (21) found 2.46-2.71% urea in the blood of sharks and somewhat less in the muscle and liver. Baglioni (26) suggests that this is very important as the presence of urea in these animals is necessary for the life processes of the heart and probably all the organs and tissues. No other results of the analysis of tissues by the new and accurate methods have been reported except those of Marshall and Davis on the dog (15).

The Urea Content of the Blood and Tissues of Various Species.

Experimental.

In the present series of experiments we have analyzed the urea content of the blood and tissues of various species of vertebrates. The animals were placed under ether anesthesia,bled,and the blood collected with a small amount of potassium oxalate added to prevent clotting. The tissues were removed,immediately placed in weighed flasks containing ethyl alcohol and again weighed. The urea content was then determined by the method of Marshall and Davis (15).

Table 1

The Urea Content of the Blood and Tissues of Various Species

Results expressed in mgms. per 100 gms of tissue

Animal	Blood whole	Blood serum	Liver	Heart	Lungs	Thigh	Abdomen	Breast	Kidney
Guinea Pig 14	26		25	22	23	17	16		104
" " 7	26		26	23	27	19	31		50
" " 21	45								152
" " 16	52								
" " A	43	42							112
Hen A	6		15	11		6		10	
" 2F	9		16	8		9		10	
" 2C	12			10		11		11	
" 2D	6			5		11		11	
Turtle		38	17			38			52
"	28								
Rabbit	60	60	65	38	40	32			155

The results of the analyses given in the above table show the variations of different species in the urea content of their blood and tissues. In agreement with the work of Bang (25),Folin and Denis (16) there is considerable variation in the urea content between different species while individual variations of normal animals of the same species tend to fall within rather definite limits. The figures for the content of the blood and tissues of the guinea pig may vary from 25-50 mgm. and the animal remain in an apparently normal condition. The data agree with those of Marshall & Davis (15) who showed that in the dog the urea was approximately equally distributed in all the tissues with the exception of the fatty tissue and the urinary tract. Thus in normal guinea pigs 7 & 14,the variations between tissues,except those of the urinary tract,were 16-31 mgms. and 19-31 mgms. respectively. On the other hand the urea content of the kidneys,due presumably to contamination of the kidney tissue with urea,was 104 & 50 mgms. respectively. The occurrence of considerable amounts of urea in turtle blood as a representative of the lower vertebrates,is also of interest. In this connection it is worthy of note that urea has been found uniformly distributed in amounts varying from 20-30 mg. per 100 gm. of tissue in the fresh water crab (cambarus virilis) (27).

The results of the analysis of the blood of the hen are somewhat lower than those obtained by Folin (16) as he obtained 8mgm. urea N. per 100cc. blood. These higher results may be due to the fact that he used a different method of analysis. In contrast to that of other animals the urea content of the tissues of the hen are low and the kidneys uniform with that of other tissues,which is in agreement with the fact that urea is not the end-product of N metabolism in the hen.

The Urea Content of the Tissues of Guinea Pigs in Starvation and on Certain Restricted Diets.

When guinea pigs were kept in the laboratory on an exclusive diet of oats it was noticed that they began to lose weight, and after a period from 15-30 days refused to eat and died. On autopsy they showed various hemorrhages in the body, especially in the subcutaneous layer knee joints, ribs and epiphyses, stomach, together with a degeneration of the bone marrow. Like conditions have been described by Holst and Frölich (22) in experiments with diets of similar character. These investigators believed the condition to be one resembling human scurvy. In order to further investigate the nature of this disturbance, guinea pigs were put on the following diets: Oats exclusively, oats and cabbage, oats and sodium citrate, oats and oranges. Water was available at all times. The animals were kept in metabolism cages and weighed at the same hour on alternate days. When the pigs showed disease symptoms and refused to eat, they were placed under ether anesthesia and bled to death, and an analysis made of their blood and tissues. Cabbage was added to the oat diet to make a normal diet by having green food present and to show that death was not caused by laboratory conditions. Sodium citrate was added as an alkali on the assumption that the disease might be of the nature of an acidosis as indicated by the work of Morgan & Beger (23). As oranges are known to be of value in the treatment of human scurvy by supplying some necessary substance of unknown nature, the so called vitamine or accessory substance, they were added to the oat diet to see if they could supply the substance lacking in the oats. The results of the analysis of blood and tissues are given in the following table:

Table 2

Urea Content of Blood & Tissues of Guinea Pigs on One-sided Diets & Starvation

Results expressed in mgms.

No.	Weights first gms.	Weights last gms.	Duration days	Blood	Liver	Lungs	Heart	Thigh	Abdomen	Kidneys	Diet
14	455	435	28	26	26	23	22	17	16	107	Oats & Cabbage
7	600	600	30	26	29	27	23	20	31	51	" "
A				47						113	
19	425	365	24	45						152	Oats & Orange
21	455	425	51	45	49	42	45	46	46	125	" " "
16	640	525	6	52							"&.45gm. Na-citrate per day
17	645	550	9	163	122	148	152	141	129	153	" " "
B				166			114			114	Oats
C				48	56	30	44				"
D	745	505	30	158	126			122	114	277	"
5	755	455	18	125	43	52	61	88	82	156	"
6	480	285	15	190	25		34			61	"
10	480	345	20	165	115	74	63	85	117	86	"
13	550	345	6	90	72	53		53	75	164	"
15	705	555	6	184	167	187	184	168		197	Inanition
25	575	490	2	48	43	47	56	50	50	130	"(no water)
24	590	455	6	96	91	90	117	101		208	Inanition
27	380	295	3	68	57	68	66		69	190	"
26	485	340	5	43	46		42	42	45	62	" (fed 20-40cc water per day)

The pigs on the exclusive oat diet showed a remarkable increase in the urea content of the blood and tissues. Approximately all the animals showed on autopsy the characteristic hemorrhages. The autopsy findings are given in complete form with the weights in the appendix. The pigs on the oat and cabbage diet remained in good condition and the urea content of blood and tissues was on analysis normal. While the results of the experiments with sodium citrate are not very definite,we were unable to obtain any apparent beneficial result,but on the contrary the results would seem to be capable of the opposite interpretation. The oranges added to the diet apparently had a beneficial effect. Pigs 19 and 20 died on the diet,but did not show the characteristic autopsies nor the high urea content of the blood and tissues. Pig 21 lived 51 days,apparently normally with no decrease in weight and when killed at that time the urea content of the blood and tissues was normal. Fasting animals show the same rise in urea content as the animals on the oat diet. Bang (24) has obtained the same results on analyzing blood from rabbits that have starved for short periods of time (2-5 days). He has attributed this increase to urea retention due to the fact that the kidneys do not excrete the normal amount of urine. We have observed that the kidneys are often no higher than the other tissues in their urea content which fact would seem to support the idea that we are dealing with a retention and not an overproduction of urea. Bang found on giving water by sound that the urea content was lowered nearly to the normal figure. We have been able to confirm Bang's results to a certain extent altho our increases in the urea content of the blood and tissues of the guinea pig are not as striking as those he obtained with rabbits in which he observed an increase amounting to nearly double on the first or second day of total fasting. Pig 25 fasted 48 hours

with no water and with that period of fasting the urea content of the tissues was not above normal. Pig 24 fasted 6 days with water present in the cage. His figures show an appreciable increase in urea. Pig 26 fasted for 5 days and was given 20-40cc of water a day thru the mouth. The urea content of its tissues was normal altho it fasted the same length of time as Pigs 15 and 24,which had an increased urea content. Pig 27 showed a smaller increase after fasting 3 days.

We do not believe that the high urea figures in our experiments on an oat diet can be ascribed to starvation alone since three animals were killed as soon as it was observed that no food was ingested In no case could the animal have starved more than 24 hours,in most cases less than this. Moreover,starving animals fail to show the characteristic autopsy findings obtained in animals on an exclusive oat diet. The analytical results in starvation and in one-sided diets may be similar but can hardly be attributed to the same cause - total starvation.

Urea Content of Hens Under Experimental Conditions.

In order to determine whether urea had any relation to the intermediary metabolism of protein in the avian organism,e.g.,hen,alanine was injected intramuscularly,the hens allowed to remain 2-5 hours and,after being placed under ether anesthesia,bled to death and the blood and tissues analyzed for urea. Urea was also injected to show the distribution in the body of the hen. The results are given in the following table:

Table 3.

Analysis of Tissues of Hens Injected with Urea and Alanine.

Results expressed in mgms.per 100 gms.tissue.

No.	Amount Injected	Hrs.after Injection	Blood Whole	Blood Serum	Liver	Breast	Thigh	Heart	Kidneys	Lungs
C	2.5gm alanine	2 hrs.	4		10	10	7	7		4
D	"	3 "	4		13	9	8		7	
E	"	5½ "	7							
2E	"	3 "	5	4						
B	2gm urea	2 "	77	83	31	45 #194	49		72	

#On the side injected.

The results show that when urea is injected it is transferred to all tissues of the body altho apparently more slowly than in other mammals (15). When alanine is injected there is no increase in the urea content of the tissues. This may be interpreted that urea plays no important part in the transformation of the amino acids to the final excretory product, uric acid, in the metabolic processes of the hen.

From tables 1 & 3 it is seen that the urea content of the kidney of the hen is no higher than that of the rest of the tissues, which indicates that the kidney in the hen is not a specialized excretory organ for urea as in other vertebrates.

In table 1, analyses of rabbit A, and in table 3, of hens B & 2E, show that the distribution of urea is approximately equal between the serum and corpuscles of the blood which confirms the results found by Bang (24)

and older investigators.

SUMMARY.

1- Values are given for the normal urea content of the blood and tissues of a number of species of vertebrates.

In vertebrates whose end-product of N metabolism is urea, the kidneys are much higher in urea as they are saturated with urine, while the urea content of other tissues is about the same as the blood.

2- During starvation or on certain restricted diets, i.e. oats exclusively, the urea content of the blood and tissues of the guinea pig is greatly increased, probably due to an abnormal retention of urea in the system.

3- Hens injected with alanine do not show any increased amount of urea in the blood and tissues, indicating that urea is not one of the stages in the intermediary N metabolism of the hen. The kidneys of the hen have the same urea content as the other tissues, showing that urea is not present to any considerable extent in the kidney excretion of the hen.

4- Urea readily distributes itself on injection to all tissues of the body. The corpuscles and serum of the blood contain approximately the same amount of urea.

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22- Holst & Frölich,Z.f.Hygiene,'12,72:1
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24- Bang,Biochem.Z.'15,72:119
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APPENDIX.

Below are given more complete protocols of the autopsies, diets, duration of experiments, and weight of the animals on alternate days.

Guinea Pig No.7.

Diet: Oats and cabbage.

Exp.begun Dec.18 - Bled to death Jan.18 - 30 days.

Animal normal.

Wts: 600.545.550.565.560.525.495.455.460.465.525.525.550.560.475.525.600.

No.14.

Diet: Oats and cabbage.

Exp.begun Jan.10 - Bled to death Feb.7 - 28 days.

Animal normal.

Wts: 455.435.450.450.465.460.465.465.485.470.475.440.435.

No.19.

Diet: Oats and orange

Exp.begun Feb.15 - Killed Mar.10 - 24 days.

Became drowsy at end of experiment and refused to eat the orange.

Autopsy: Stomach; two pinpoint ulcers. No subcutaneaous hemorrhages. Slight indication of hemorrhages at end of ribs.

Wts: 425.450.450.445.460.465.465.450.460.455.455.415.365.

No.20.

Diet: Oats and orange.

Duration: Feb.15-Mar.7. Dead in cage. 21 days.

Lost weight steadily despite the ingention of food. Ate oats and orange on March 6.

Autopsy: Hemorrhages (?) in sternum and ribs. No subcutaneous or knee hemorrhages. Lymph gland in groin enlarged, left side hemorrhagic, Stomach normal.

Wts: 405.395.385.385.375.365.355.340.320.305.280.

No.21.

Diet: Oats and oranges (45 gm sodium citrate per day from Feb.23 - Mar.12.)

Duration: Feb.15-Apr.4. Killed. 51 days.

Animal normal.

Wts: 455.455.455.455.455.470.450.465.450.445.440.440.440.425.440.415.
420.420.425.435.435.420.430.415.425.

No.16.

Diet: Oats and sodium citrate. (.45gm per day) Citrate given in about 10cc water with a medicine dropper. Would not eat boiled cabbage; oats given on third day.

Duration: Feb.15-21. Killed. 6 days.

Animal refused to eat only on last day. It had clonic contractions, partially paralyzed in hind legs.

Autopsy: Normal,(Minor hemorrhages in bones not looked for.)

Wts: 640.575.560.525.

No.17.

Diet:

Duration: Feb.12-21. 9 days.

Ate normally to within 24 hours before killing the animal.

Autopsy: Stomach full of ulcers (hemorrhagic). Slight hemorrhages around knee joints and epiphyses. No subcutaneous hemorrhages.

Wts: 645.665.635.585.550.

No.18.

Diet: Oats plus citrate (.3gm) first 6 days; oats only for 8 days.

Duration: Feb.15-29. Found dead in cage.

Animal died very suddenly without showing any apparent symptoms.

Autopsy: Hemorrhages around sternum,ribs and knee joints. Small pinpoint ulcers in stomach and one large one. Hemorrhages in lymph

glands in groins.

Wts: 445.465.465.455.425.385.

No.5.

Diet: Oats.

Duration: Dec.18-Jan.5. Killed. 18 days.

Autopsy: Marked hemorrhages in knees and ribs - a few subcutaneous. Lungs congested and hemorrhagic. Muscle,especially heart and abdominal,peculiar dark red color.

Wts: 755.710.670.655.690.650.605.550.495.455.

No.D.

Diet: Oats.

Duration: Nov.17-Dec.17. Killed. 30 days.

Autopsy. Not noted.

Wts. 745.715.720.700.700.710.725.730.735.720.715.720.735.670.615. 550.525.505. (Some weights taken every day.)

No.6.

Diet: Oats.

Duration: Dec.18-Jan 2. Killed. 15 days.

Autopsy: Marked hemorrhages around knee joints and the epiphyses. Lungs slightly hemorrhagic. Right kidney has a cyst.

Wts: 480.430.385.345.355.340.320.290.285.

No.10.

Diet: Oats.

Duration: Dec.18-Jan.7. Killed. 20 days.

Autopsy: Marked hemorrhages in subcutaneous layer,knee joints,ribs and epiphyses. Stomach a mass of ulcers and hemorrhagic. Pregnant uterus hemorrhagic.

Wts: 480.465.445.470.465.445.475.455.420.375.345.

No.13.

Diet: Oats.

Duration: Jan.8-Feb.1. Killed. 24 days.

Autopsy: Typical hemorrhages.

Wts: 550.505.530.535.505.520.515.485.470.450.430.405.390.370.345.

(Some weights taken every day.)

No.9.

Diet: Oats.

Duration: Dec.18-Jan.8. Died. 21 days.

Autopsy: Typical hemorrhages,especially hemorrhagic ulcers in the stomach. (Legs seemed paralyzed the day before.)

Wts: 760.685.700.695.745.740.735.730.705.565.525.

No.11.

Diet: Oats.

Duration: Jan.8-Jan.29. Died. 21 days.

Autopsy: Typical hemorrhages.

Wts: 710.685.675.669.615.655.675.645.585.560.525.500.460.

No.13.

Inanition: (Oats present in cage but none eaten.)

Duration: Feb.15-Feb.21. Killed. 6 days.

Autopsy: Liver full of cocidia; otherwise normal.

Wts: 705.645.595.555.

No.25.

Inanition: (No water.)

Duration: 48 hours.

Autopsy: Normal.

Wts: 575.525.490.

No.24.

Diet: Inanition. (Water present in cage.)

Duration: Apr.25-May 2. Killed. 6 days.

Autopsy: Normal.

Wts: 590.555.520.505.485.470.455.

No.26.

Diet: Inanition. Fed 20-40cc water thru the mouth each day.

Duration: Apr.26-May 2. Killed. 5 days.

Autopsy: Normal.

Wts: 485.460.435.400.350.340.

No.27.

Diet: Inanition. Water present in the cage.

Duration: May 3-May 6. Killed. 3 days.

Autopsy: Normal.

Wts: 380.335.310.295.